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Developing Breast Cancer Program at Xavier; Genomic and Proteomic Analysis of Signaling Pathways Involved in Xenohormone and MEK5 Regulation of Breast Cancer

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14. ABSTRACT- Xavier University (XU) and the Tulane Cancer Center (TCC) will build a core of human talent that will address scientific problems such as drug resistance and the effect of environmental agents on breast cancer (BC) in the African-American community. A multi-part research and training program will generate data, develop new research programs and train new faculty and African-American students in BC research. The first component will fund two research projects. The Wang and Burow project will elucidate a previously unexplored cellular signaling mechanism that leads to drug resistance in breast carcinoma cells derived from African American women and women of other ethnicities. The Wiese and Hill project will identify and characterize the genes and gene products associated with BC cell proliferation induced by exposure to pesticide mixtures and is relevant to the African American community in Southern States where pesticide exposure is relatively high. The second part of the program aims to increase the number of faculty at XU involved in BC research by supporting two junior faculty members to develop BC research projects with a TCC mentor. The third objective will support research training of XU undergraduates and pharmacy students. The fourth objective will provide workshops, seminars and research opportunities in BC research for the XU community. This program will enhance the understanding of unique aspects of BC development and progression among African American women and will contribute to the elimination of the "mortality gap" between African-American BC patients and women of other ethnicities.

15. SUBJECT TERMS- MCF-7, MEK5, Environmental Estrogens, pesticides, Proteomics

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Introduction

African American women are at higher risk for breast cancer (BC) mortality compared with their white counterparts. Over the past decade BC mortality has decreased 1%-2% per year in white women, but not in African-American women. The resulting "mortality gap" is a serious national problem, and understanding the reasons for it and developing solutions must be a high priority. Thus, BC research must focus on developing breast cancer models that would aim to accurately predict the disease development and progression among African-American women. ***We are convinced that increasing the involvement of African American students in BC research will greatly contribute to increasing awareness of the disease in the African American community, which in turn will increase the likelihood of early detection of the disease. Furthermore, the focus on the unique aspects of BC in African American women will lead to better understanding of the disease, and to better treatment options for African American women. This will eventually minimize or eliminate the BC "mortality gap".*** To this end we are developing a training program at Xavier University of Louisiana (XU) in collaboration with the Tulane University Cancer Center (TCC). More than 90% of Xavier's student body is African American has active programs (MBRS, MARC, RISE, NSF/MIE) designed to increase the number of minority students pursuing careers in medical and biomedical research. Through this BC training program, African American students will have the opportunity to become involved in BC research. Tulane (TU) and Xavier have a long history of collaborations involving joint centers and programs and individual collaborations between Tulane and Xavier faculty and staff are common. This new initiative will provide funds for yet another collaboration offering a unique opportunity for XU researchers to establish a BC research program for the benefit of XU students and, eventually, the African-American community. The goals of the training program are to create an environment that fosters BC research, in which XU investigators will receive substantive training and to complete substantive research projects of high relevance to the eradication of BC. The program will enable XU investigators to publish their results in peer-reviewed literature and advance toward independently funded BC research programs. The program includes two full research projects that involve an XU researcher and a qualified TCC mentor. The program will identify two additional XU researchers who have expressed an interest in BC research but do not have prior funding in BC. Participating XU faculty will get the opportunity to network and learn about BC research through participation in the TCC weekly seminar program and the signal transduction workshop that will focus on breast and prostate cancer. The two additional XU faculty involved will develop a mini-proposal in Y1-2 and carry out pilot studies with the advisory of a mentor faculty from TCC in Y2-4. The results of all program research studies will be used as a basis for future proposals in the area of BC. Yearly symposia will be held to provide information to XU students and faculty as well as to enrich the experience of the participating members regarding research opportunities in BC. Multiple project group meetings will be held each year to discuss current data, manuscripts in preparation, funding opportunities and issues regarding project operations.

Year Five Progress Summary

The main goal of this program in Y5 was to focus on completing and expanding program aims as well as submitting publications and research proposals building on this work. The project #1 team continued a high level of productivity submitting one manuscript for publication that was accepted and published in Breast Cancer Research and submitted three grant proposals. The project #2 team carried out a massive PCR array study collecting gene data on all pesticide mixtures and controls previously evaluated in the breast cancer proliferation studies in Y1-3 of the program. The two new pilot projects each continued to develop significant data. The cancer research programs at Xavier continued to develop and expand. The DOD Prostate Cancer

program that started in 2004 ending with both pilot projects continuing with new sources of funding, the NCI P20 program that started in 2005 continued to develop two cancer pilot projects in health disparities research and trained students with research experiences and the cancer biology course. Cancer research support programs continue to be developed on campus through Xavier involvement in the Louisiana Cancer Research Consortium (LCRC). The Xavier LCRC has supported the hire of 6 new cancer research faculty with startup packages. The LCRC continues to support the development of cancer research resources on campus including instrumentation, research support personnel and regular cancer research seminars and meetings of cancer researchers on campus. This cancer research focused environment on campus has spawned the development and submission of an NIH NCRR RCMI grant focused on developing cancer research core facilities and pilot projects. The Xavier RCMI grant was submitted in January 2009 under the leadership of Dr. Guangdi Wang, lead investigator of project #1 in this DOD BC program.

Request for No Cost Extension After Y5

While all research projects are addressing aims and goals, we plan to file a request for a no cost extension to complete all project aims. To fund this effort, we will use carry over funds that have accumulated through the regular funding period. Details on the goals of this no cost extension period are included in each relevant section below and are also detailed on a separate no cost extension request.

Body

Task 1

Complete two substantive research projects of high relevance to eradication of breast cancer

Project 1

"Chemoresistance in Breast Carcinoma Cells: MEK5-BMK/Erk5 Expression and Proteomic Analyses"

Guangdi Wang, Ph.D., Department of Chemistry, Xavier University of Louisiana PI (Trainee)
Mathew E. Burow, Ph.D., Department of Medicine, Tulane University School of Medicine (Mentor)

Aim 1: To demonstrate the requirement for and the role of the MEK5 pathway in survival signaling and suppression of apoptosis in MCF-7 breast carcinoma cells.

(1). Implicate MEK5 activation in cell survival signaling, prevention of anti-estrogen and chemotherapeutic drug-induced cell death using MCF-7 stable, transiently transfected cells and ZR-75-30. (Months 1-18).
(2). Implicate apoptotic suppression as a mechanism for MEK5-mediated survival and drug-resistance (Months 12-24).

Year Five Progress (April 19, 2008-April 18, 2009)

An NIH-R01 was submitted in 2008:
"MEK5-Erk5 pathways in survival signaling and tumor progression to drug resistance"

(M. Burow Principal Investigator) 25% effort
Agency: NIH-NCI (BMCT), Type: 1**R01CA**125806-01A1, 07/01/09-06/30/14, $1,856,250
The long-term goal of this project is to identify the signaling pathways critical to the development of resistance to chemotherapeutics agents and the progression to a hormone independent phenotype in carcinoma of the breast.
Submitted July 2008 - **(scored 219)**

Aim 2: To characterize differences in protein expression between MCF-7N (APOP-Sensitive), MCF-7M (APOP-Resistant) and ZR-75-30 breast carcinoma cells and identify anti-apoptotic proteins, such as Survivin, within MEK5-expressing cell lines.

(1) Prepare samples for 2D gel separation. (Months 18-24).
(2) Separate proteins on 2D gel electrophoresis, compare differences in protein expression, and perform in-gel tryptic digestion of excised protein products. (Months 24-36).
(3) Sequences obtained from tryptic digests will be used to characterize and identify protein expression differences between drug resistant ZR-75-30 and MCF-7 breast carcinoma cells with a focus on known anti-apoptotic proteins or novel apoptotic domain containing proteins (BCl-2 homology (BH), baculovirus IAP repeat (BIR0, caspase activation recruitment domain (CARD), etc.). (Months 24-36).

Year Five Progress (April 19, 2008-April 18, 2009)

Overview

1. An abstract was presented at the DOD Era of hope 2008 meeting
2. Wang's lab and Burow lab worked closely to revise the original manuscript, design and carry out additional experiments needed to improve the manuscript, and finalize the paper for publication in December of 2008. The manuscript has now appeared in the journal Breast Cancer Research:
   Zhou C, Nitschke AM, Xiong W, Zhang Q, Tang Y, Bloch M, Elliott S, Zhu Y, Bazzone L, Yu D, Weldon CB, Schiff R, McLachlan JA, Beckman BS, Wiese TE, Nephew KP, Shan B, **Burow ME**, **Wang G**. Proteomic analysis of tumor necrosis factor-alpha resistant human breast cancer cells reveals a MEK5/Erk5-mediated epithelial-mesenchymal transition phenotype. *Breast Cancer Research* **2008**;10(6):R105. Epub 2008 Dec 16.
3. Wang and Burow worked together to prepare a proposal for DOD BC Idea titled "MEK5-Erk5 Signaling Regulates Epithelial-to-mesenchymal Transition in Breast Cancer Progression". The proposal was submitted to DOD BC Idea in May 2008, was reviewed and not funded.
4. Wang and Burow worked together to prepare a 4-year proposal for NIH titled "Proteomic and Phosphoproteomic Study of MEK5-Erk5 Signaling and Rapid Estrogen Signaling in Breast Cancer Cells". The proposal was submitted to NIH's SCORE program as an SC-1 type on January 25, 2009.
5. As the proposed Program Director, Guangdi Wang prepared and submitted an institutional program proposal entitled "Xavier's RCMI Cancer Research Program" to NIH's NCRR division on January 25, 2009.

Era of Hope 2008 Abstract:

Proteomic Characterization of MCF7 Human Breast Cancer Cells Resistant to TNF-alpha and Chemotherapeutic Drugs

Changhua Zhou[1,8], Wei Xiong[7], Qiang Zhang[1], Yan Tang[4-7], Michael Bloch[3-5], Christopher B. Weldon[3-5], Steven Elliott[3-5], John A. McLachlan[3-6], Barbara S. Beckman[3-6], Thomas E.Wiese[2], Matthew E. Burow[3-7], and Guangdi Wang[1]

1. Department of Chemistry, 2. College of Pharmacy, Xavier University of Louisiana, New Orleans, LA 70125, 3. Department of Medicine, Section of Hematology& Medical Oncology, 4. Center for Bioenvironmental Research, 5. Tulane Cancer Center, 6. Department of Pharmacology, 7. Department of Surgery, Tulane University School of Medicine, New Orleans, LA 70112, 8. Department of Immunology, Sichuan University, Chengdu, China

Despite intense studies on the mechanisms of chemotherapeutic drugs resistance in human breast cancers, few reports investigate systematically the possible mechanisms responsible for the resistance to TNF-α, a sensitizing agent in tumor chemotherapy. In this study, 2-DE and LC-MS/MS approaches were used to compare the differences of protein expressions between an MCF-7 breast caner cell line resistant to TNF-α and its parent MCF-7 cells sensitive to TNF-α. Proteomic analysis identified seven protein spots that differed significantly in abundances in the two cell lines. The differential expressions of these protein spots were verified with both semi-quantitative RT-PCR and real-time RT-PCR assays, and the genes e-cadherin, β-catenin, snai1, slug and δ-ef1 involved in the epithelial-mesenchymal transition (EMT) were detected simultaneously. The study suggests that upregulations of Vimentin (VIM), Heat shock 70 kDa protein 4 (HSPA4), Glutathione S-transferase P (GSTP1), and Creatine kinase B-type (CKB), and downregulations of Keratin 8 (KRT8), Keratin 19 (KRT19) and Glutathione S-transferase Mu 3 (GSTM3), and the epithelial to mesenchymal transition are possibly related to TNF-α resistance in MCF-7 cells.
Keywords: Breast cancer, TNF-α resistance, Drug resistance, 2-DE, Mass spectrometry, Proteome analysis, EMT

Abstract of the published article in *Breast Cancer Research, 2008;10(6):R105:*

Proteomic analysis of tumor necrosis factor-alpha resistant human breast cancer cells reveals a MEK5/Erk5-mediated epithelial-mesenchymal transition phenotype.

Abstract

Introduction: Despite intensive study of the mechanisms of chemotherapeutic drug resistance in human breast cancer, few reports have systematically investigated the mechanisms that underlie resistance to the chemotherapy-sensitizing agent tumor necrosis factor (TNF)-a. Additionally, the relationship between TNF-a resistance mediated by MEK5/Erk5 signaling and epithelial-mesenchymal transition (EMT), a process associated with promotion of invasion, metastasis, and recurrence in breast cancer, has not previously been investigated.

Methods: To compare differences in the proteome of the TNF-a resistant MCF-7 breast cancer cell line MCF-7-MEK5 (in which TNF-a resistance is mediated by MEK5/Erk5 signaling) and its parental TNF-a sensitive MCF-7 cell line MCF-7-VEC, two-dimensional gel electrophoresis and high performance capillary liquid chromatography coupled with tandem mass spectrometry approaches were used. Differential protein expression was verified at the transcriptional level using RT-PCR assays. An EMT phenotype was confirmed using immunofluorescence staining and gene expression analyses. A short hairpin RNA strategy targeting Erk5 was utilized to investigate the requirement for the MEK/Erk5 pathway in EMT.

Results: Proteomic analyses and PCR assays were used to identify and confirm differential expression of proteins. In MCF-7-MEK5 versus MCF-7-VEC cells, vimentin (VIM), glutathione-S-transferase P (GSTP1), and creatine kinase B-type (CKB) were upregulated, and keratin 8 (KRT8), keratin 19 (KRT19) and glutathione-S-transferase Mu 3 (GSTM3) were downregulated. Morphology and immunofluorescence staining for E-cadherin and vimentin revealed an EMT phenotype in the MCF-7-MEK5 cells. Furthermore, EMT regulatory genes SNAI2 (slug), ZEB1 (d-EF1), and N-cadherin (CDH2) were upregulated, whereas E-cadherin (CDH1) was downregulated in MCF-7-MEK5 cells versus MCF-7-VEC cells. RNA interference targeting of Erk5 reversed MEK5-mediated EMT gene expression.
Conclusions: This study demonstrates that MEK5 over-expression promotes a TNF-a resistance phenotype associated with distinct proteomic changes (upregulation of VIM/*vim*, GSTP1/*gstp1*, and CKB/*ckb*; and downregulation of KRT8/*krt8*, KRT19/*krt19*, and GSTM3/*gstm3*). We further demonstrate that MEK5-mediated progression to an EMT phenotype is dependent upon intact Erk5 and associated with upregulation of SNAI2 and ZEB1 expression.

DOD Breast Cancer Idea Grant Submitted in May 2008:

Technical Abstract:
Title: MEK5-Erk5 Signaling Regulates Epithelial-to-mesenchymal Transition in Breast Cancer Progression

Background: Progression of cancer cells to a resistant phenotype is in general characterized by the acquisition of molecular or cellular changes that alter or subvert the response to therapeutic agents. Numerous studies have shown that survival factor initiated signals such as the mitogen-activated protein kinase family (MAPK) and downstream transcription factors such as STATs, NF-kB and ER play important roles in cancer cell's progression to drug resistance. EMT (epithelial-to-mesenchymal tranisition) presents another complication in cancer therapy. EMT is the process by which epithelial cells convert to mesenchymal cells and is essential in embryonic development. However, it appears that aberrant activation of EMT later in life occurs in cancer progression, and is involved in highly aggressive, poorly differentiated breast cancers with increased potential for metastasis and recurrence.

Objective/Hypothesis: The primary long term objective of this research is to understand how MEK5-Erk5 signaling controls progression of breast cancer cells to a more aggressive and resistant phenotype. We **hypothesize** that MEK5-Erk5 signaling drives expression of members of the SNAI (snail, slug) family of proteins leading to an epithelial-to-mesenchymal transition and tumorigenic phenotype. This hypothesis is supported by our preliminary studies demonstrating the role of MEK5-Erk5 signaling in promoting a tumorigenic phenotype in coordination with enhanced survival signaling and gene expression in breast cancer cell lines. Furthermore, using a proteomics approach we provide evidence that MEK5 functions through activation of downstream transcription factors, specifically SNAI2, to mediate expression of key EMT regulating genes.

Specific Aims: The proposed **Specific Aims** are designed to identify the role of SNAI2 signaling as a downstream component of MEK5-Er5 mediated breast cancer cell tumorigenesis and epithelial-to-mesenchymal transition
Specific Aim 1: To demonstrate the requirement for and role of the MEK5-Erk pathway in survival signaling and tumorigenesis in cancer cells.
Specific Aim 2: To test the hypothesis that SNAI2 expression is required for MEk5-Erk5 mediated epithelial-mesenchymal transition.
Specific Aim 3: To test the hypothesis that constitutive SNAI2 expression promotes EMT and tumorigenesis.

Study Design: The proposed specific aims are intended to elucidate the mechanism by which MEK5-Erk pathway in survival signaling and tumorigenesis regulates EMT. Multiple cell lines will be used to ensure consistency across cell lines for experiments designed to test the hypothesis that MEK5-Erk5 signaling mediated expression of SNAI is implicated in EMT. Specific Aim 1 seeks evidence that shows that MEK5-Erk pathway is required to mediate expression of key EMT regulating genes. Specific Aim 2 will focus on the tests to show the requirement of SNAI2 as a downstream protein. Specific Aim 3 will further test if stable transfection of SNAI2 gene indeed promotes EMT and tumorigenesis. All studies will be conducted in vitro using the Boyden chamber assays for migration, flow cytometry and proliferation assays to study enhanced proliferation, and apoptosis assays to determine survivability. Coculturing will be utilized as well as conditioned media from hMSC to determine the nature of the interaction between hMSC and breast tumor cells. Inhibitors along with shRNA to SDF-1, CXCR4, and ER will be used in conjunction with their appropriate control vehicles or non-silencing shRNA.

Abstract of SC-1 proposal submitted to NIH on January 25, 2009
Title: Proteomic and Phosphoproteomic Study of MEK5-Erk5 Signaling and Rapid Estrogen Signaling in Breast Cancer Cells
PI: Guangdi Wang, Ph.D., Xavier University of Louisiana

SUMMARY

Approximately 30-40% of all breast cancers are estrogen receptor-negative (ER-) and are non-responsive to anti-estrogen treatment. These breast tumors are also more aggressive and have a higher recurrence rate. However, while they are hormone independent, ER (-) breast tumors respond to estrogen stimulation in their progression and survival via nongenomic signaling pathways. The long term goal of this project is to understand the signaling network involved in a breast cancer cell line that has been transformed from a parental, estrogen dependent, epithelial carcinoma cell line (MCF-7) into hormone independent, mesenchymal phenotype (MCF-7-MEK5) driven by the constitutive activation of MEK5-Erk5 signaling cascade, and to identify new therapeutic targets for the ER independent estrogen pathways. We hypothesize that specific proteomic techniques for phosphorylated protein identification and quantification can reveal unbiased global proteomic differences in breast cancer cells that differ in resistance to hormone therapy and estrogen signaling pathways. We further hypothesize that rapid estrogen signaling in MCF-7-MEK5, an ER (-) breast cancer cell line variant is regulated by a non-ER mechanism. To test these hypotheses we propose the following specific aims: Aim 1: To develop specific proteomic techniques for identification and quantification of low abundance and phosphorylated proteins that underlie the fundamental differences in signaling pathways between MCF-7 and MCF-7-MEK5 breast cancer cell lines. Aim 2: To investigate the rapid estrogen signaling pathways in MCF-7-MEK5 cells by a phosphoproteomic approach. Achieving this aims will allow us to 1) elucidate the rapid signaling pathways in a breast cancer cell line where loss of ERα expression is driven by the constitutive activation of MEK5-Erk5 signaling cascade, 2) identify unique protein targets involved in the MEK5-Erk5 mediated rapid estrogen signaling in a more aggressive breast cancer phenotype, and 3) identify common downstream pathways for ER(+) (MCF-7) and ER(-) (MCF-7-MEK5) as potentially more effective therapeutic targets.

Abstract of RCMI proposal submitted to NIH on January 25, 2009

Xavier's RCMI Cancer Research Program(New Application)

Program Director: Guangdi Wang, Ph.D.,

In the past decade, Xavier University of Louisiana has embarked on an ambitious, strategic initiative to enhance its research competitiveness in biomedical fields. Our long term goal is to, in the next 5 to 7 years, be seen nationally not only for our eminence in graduating African American science, pre-med and pharmacy students, but also for national prominence in cancer research. Since 2002, in spite of the destruction rendered by Katrina, Xavier has made significant advances toward achieving this goal through the establishment of both the Center for Minority Health Disparities, launched in 2002, and through membership in the Louisiana Cancer Research Consortium. Xavier researchers have received significant external funding for cancer research from the National Institutes of Health, Department of Defense, National Cancer Institute, and Louisiana Board of Regents, among others. To determine what next steps needed to be taken to build on the above-described progress and take Xavier "to the next level," a comprehensive RCMI planning process was carried out through faculty and staff surveys, focus groups, and forums, with the assistance of a variety of external advisors. As a result, barriers to competitiveness and strategies to overcoming them were identified in such areas as recruiting and retaining research competitive faculty, infrastructural support for biomedical research, and lack of centralized core laboratories with supporting staff. To address these barriers, this proposal requests funding to assist Xavier in: (1) increasing the number and quality of cancer researchers and providing them with start-up packages, release time, and mentoring opportunities; 2) enhancing the competitiveness of existing research faculty and programs by providing seed funding for developmental projects, enhanced professional development opportunities and increased access to postdoctoral associates and research staff; (3) developing three research instrumentation cores; (4) offering enhanced administrative services to assure research support activities meet faculty needs, and (5) supporting selected pilot projects that will assist investigators who are at the "cusp" of becoming fully competitive.

Aim 3: In this task we will use RNA interference strategies to validate a role for the Erk5 pathway in downstream gene expression and in suppression of chemotherapeutic drug-induced apoptosis. Our preliminary analysis revealed survivin expression was increased in drug-resistance and MEK5 expressing breast carcinoma cells. Subsequently we will characterize the role of these downstream targets such as Survivin, in suppression of apoptosis and drug-resistance.

(1) Optimize pSUPER base RNA interference (RNAi) suppression of ERK5 expression in breast carcinoma cells (Month 15-18).
(2) Confirm a role for Erk5 signaling in MCF-7N-CA-MEK5, and MCF-7M-(RESIST) cell survival using pSUPER-Erk5-RNAi. (Months 18-28).
(3) Develop/validate RNAi strategies for Survivin suppression using pSUPER method as above. Use RNAi to implicate Survivin expression in drug resistance and apoptotic signaling of MCF-7 and ZR-75 breast carcinoma cells (Months 24-36).
(4) Develop, validate and use RNAi strategies for novel targets identified from proteomic analysis of drug resistant breast carcinoma cells from Aim 2. (Months 36-48).

No Progress on this Aim in Year 5

No Cost Extension Goals:
1. Further elucidate the signaling network involved in the transition of an apoptosis-sensitive epithelial breast cancer cell type to a mesenchymal, estrogen independent phenotype through:
   a. Proteimics analysis of constitutively expressed phosphoproteomic differences between MCF-7N and MCF-7MEK5
   a. Proteimics analysis of alterations in the phosphoproteomes in MCF-7N and MCF-7-MEK5 upon estrogen stimulation. The goal of these experiments is to further elucidate the signaling network involved in the transition of an apoptosis-sensitive epithelial breast cancer cell type to a mesenchymal, estrogen independent phenotype.
2. Develop and optimize experimental protocols for phosphoprotein enrichment, image visualization, and mass spectrometric analysis.
3. Prepare a manuscript based on phosphoproteomics results obtained from the proposed experiments.

Project 2
Interactions of estrogen and progestin active environmental chemicals on BC cell proliferation, survival and gene expression

Thomas E. Wiese, Xavier University College of Pharmacy PI (Trainee)
Steven R. Hill, Tulane University School of Medicine (Mentor).

Year Five Progress (April 19, 2008-April 18, 2009)

Research Assistant
Mr. H. Chris Segar continues as research assistant on this project.

Collaboration between Dr. Wiese at Tulane Cancer Center and Dr. Hill
Dr. Wiese has been in close contact with Dr. Hill since the start of this project through phone, email or meetings. While the project was designed to take place entirely in the Wiese lab, Dr. Hill continues to provide input on experimental design and data interpretation. The main

contribution of Dr. Hill to this project has been discussions relating to the use of microarray technology to identify specific genes or classes of genes that may be related to the observed mixture effects (see Y2 progress of Aim 2 below). Dr. Hill has also provided insight regarding the management of the overall training program (see tasks 2 and 3 below).

Preliminary and Y1-Y4 results summary

The series of pesticides included in this study included isomers and metabolites of DDT and methoxychlor. Each are known to have weak estrogen, androgen and/or progesterone activity. A series of MCF-7 proliferation studies were conducted to identify novel interaction effects of binary mixtures of these compounds. The initial studies were designed to include one pesticide at the lowest observed effect level (LOEL) and the other at the highest dose possible (10-5 M). Experiments were also conducted to determine if mixing the pesticide (high dose) with sub-optimal concentrations of estradiol-17b (E2) enhanced estrogen induced proliferation. This series of experiments did not identify mixture combinations with more than the additive cell proliferation activity expected from the compounds alone at the same concentrations. These same mixtures were examined in the MVLN estrogen responsive reporter gene assay where similar additive effects were also observed. See Y1 progress report for more information. At this point, Dr. Wiese decided to examine mixtures that contained one of the organochlorine pesticides along with one of three organophosphate pesticides. We have observed a positive sensitizing or potentiation effect of organophosphate pesticides on the weak estrogen dependant proliferation activity of organochlorine pesticides (increased potency). This action can be eliminated by antiestrogen and is likely estrogen receptor (ER) dependant. The observation that this sensitizing effect was not observed in the reporter gene system suggests that the mechanisms involved are more complex than a simple stimulation of classical ER transactivation activity. Finally, the observation of this sensitizing effect suggests a hypothesis that exposure to low levels of weakly estrogenic pesticides in combination with an organophosphate pesticide might result in more breast cancer cell proliferation than would be expected by the organochlorine alone. The organophosphate compounds in this study are known to have antiandrogen activity. Considering that androgen agonists are known to inhibit estrogen regulated processes in some cells, it is reasonable that treatment with antiandrogens may relieve such suppression, resulting in a relative increase in organochlorine induced estrogen activity. The organochlorine compounds in the study are considered persistent contaminants with long elimination half lives. Thus, chronic exposure to low concentrations may have more estrogenic activity than would be expected if cells are sensitized or stimulated by periodic exposure to organophosphate pesticides. Contamination from older pesticides that are no longer used might be more significant if one is exposed to current use pesticides. See Y1 – Y4 progress reports for more information.

Aim 1: Examine the effects of binary mixtures of estrogen and progestin active environmental compounds on cell proliferation and survival.

(a). Develop treatment mixture matrix and plan for proliferation experiments (Months 1−2).

(b). Perform cell proliferation studies with binary mixtures of pesticides (Months 1−18

(c). Identify mixtures with novel effects on cell proliferation (Months 6−20).

In Year 3, we re-established the lab after Katrina and repeated experiments used to obtain the above results. This was an effort to validate that the new cell, new reagents and new lab conditions were able to produce the same results as obtained prior to Katrina. These experiments did correlate with previous findings.

Mixture combinations that produced the most dramatic sensitization effect in the breast cancer proliferation assay were selected for PCR array analysis in Aim 2. These are: Parathion and opDDT, Fenitrothion and opDDT, and HPTE and opDDT.

Year Five Progress (April 19, 2008-April 18, 2009)

No new data was required or generated in Y5 for this aim.

Aim 2: Conduct cDNA microarrays to define a set of genes that are coordinately or differentially regulated by treatment with environmental hormones. Preparations from cells grown and exposed to mixtures of hormone active pesticides in the Wiese Lab will be evaluated for differential expression of genes in the Tulane Center for Gene Therapy.

(a). Identify target genes related to breast cancer cell proliferation from literature searches that will be used in gene array studies (Months 1−12).

(b) Prepare cells for gene array analysis after exposure to mixtures of pesticides. (Months 9−24).

(c) Run gene array analysis on cell preparations and analyze data (Months 12−36).

In Year 3 we obtained the equipment and prepared the lab for the genomic phase of this project. To obtain the equipment, Real Time PCR, a proposal was submitted to the Xavier College of Pharmacy and a BioRad iQ5 was purchased for use in the Wiese Lab for this project and subsequent use in Pharmacy teaching labs. The support of the college of provide this $36,000 instrument is significant. In addition, considerable time was spent testing RNA prep methods and developing 6 well plate cell seeding methods that would produce at least 10 ugms RNA. The Qiangen RNA prep method with the shredder and DNA removal was found to work well.

In Y4, we have completed training and application development of the PCR Array assay. Methods have been optimized to obtain pure RNA and the actually PCR Array method has been optimized and validated for precision. The technical aspects of this assay were more complex than anticipated and this process took more time than expected. However, we now have confidence that the procedure from plating and treating cells, harvesting RNA, making cDNA, loading and running PCR array plates and preliminary analysis of multiple experiments has all been worked out. Preliminary data from the PCR array analysis will be presented at the Era of Hope meeting in June 2008. Results from preliminary data suggests that there are unique gene expression profiles for the pesticide mixture treatments in MCF-7 cells in relation to the untreated and treatment with estradiol. Some PCR array single run results for and statistical analysis of multiple runs of controls (5 replicates) were shown in the Y4 progress report. It is important to note that typical estrogen regulated genes are shown to be significantly induced by the estradiol treatment in relation to the untreated cells and that the precision of the assay over multiple runs is high (p value 0.01). A preliminary plot of gene fingerprints (expression fold over control) for the pesticides and mixtures in this study was also presente in the Y4 progress report.

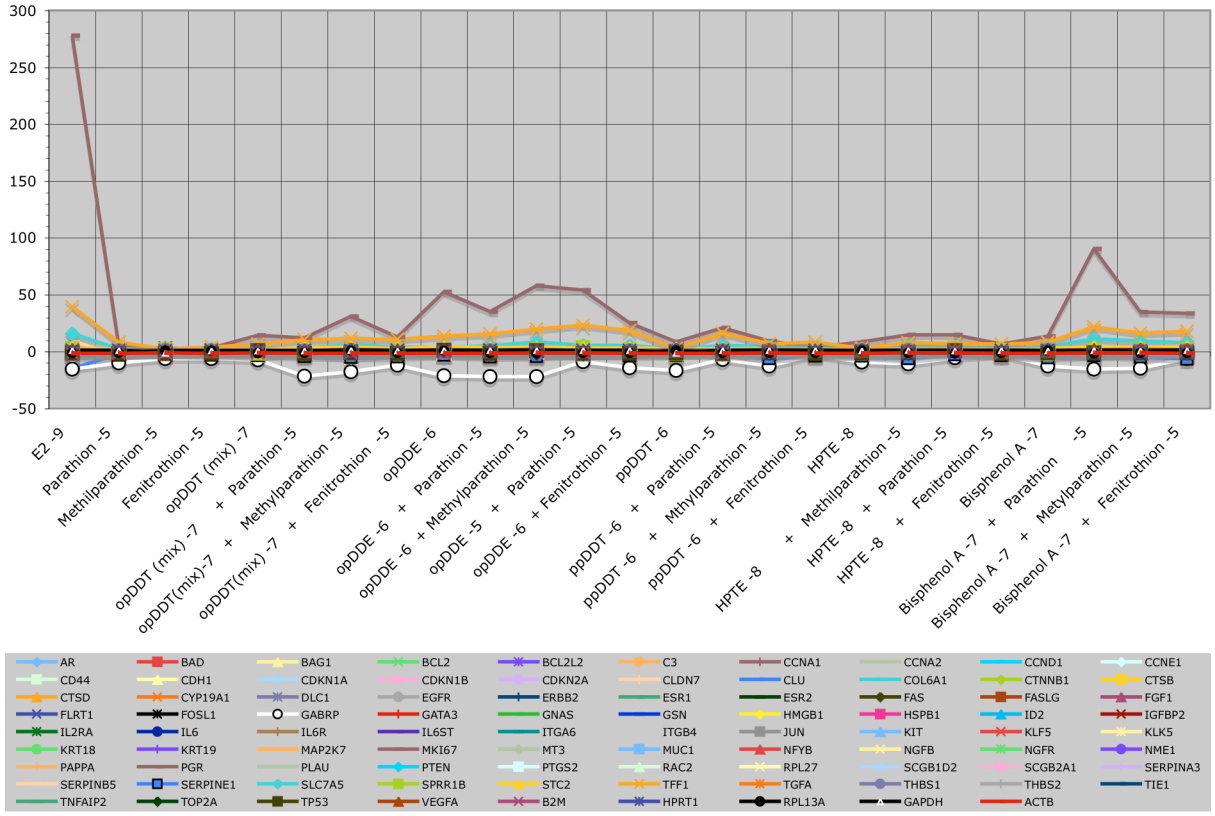
Year Five Progress (April 19, 2008-April 18, 2009)

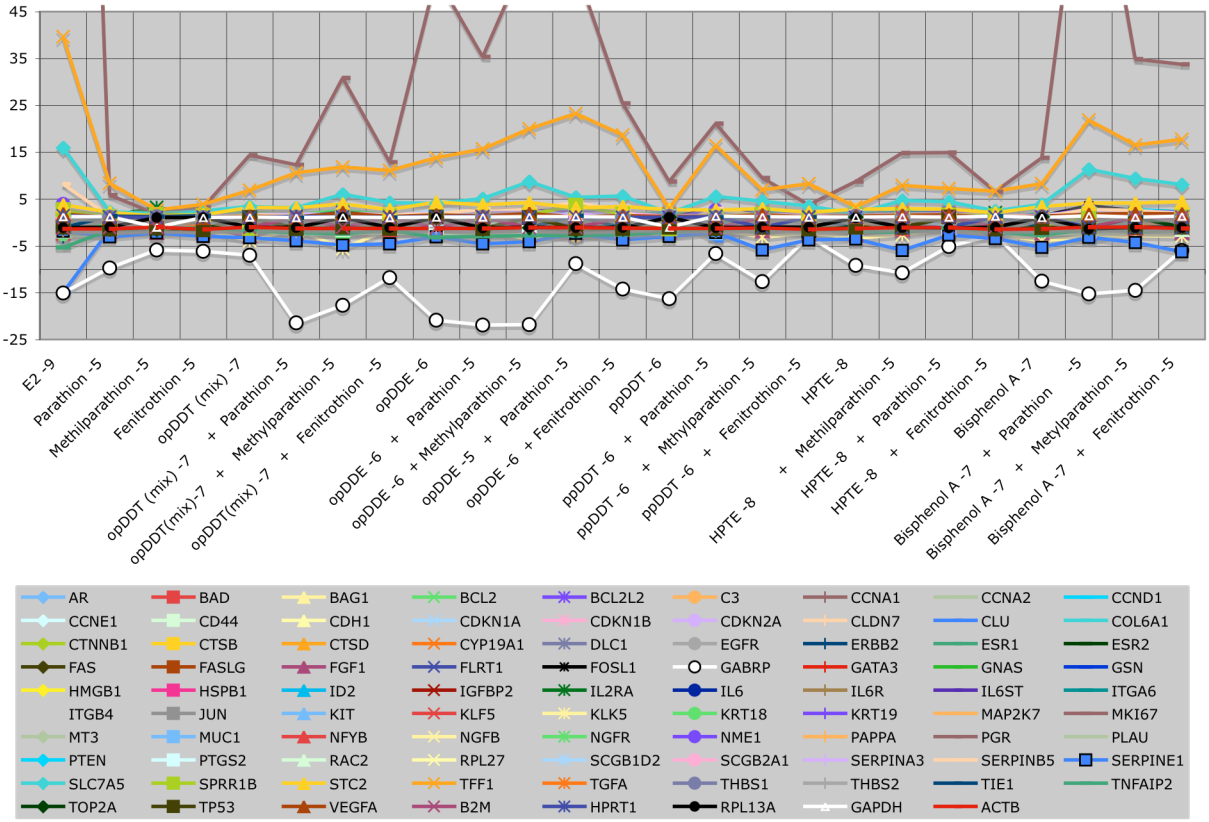
The primary goal of Y5 is to complete the PCR array analysis of all of the pesticide mixtures

evaluated in Aim #1 and then to perform data analysis to determine what genes are unique to each treatment. Due to the close structure relationship to DDT type pesticides, bisphenol A, a component of some plastics, was included in this study.

PCR Array results of all pesticide mixtures and controls from Aim 1

Results shown below are normalized fold induction plots of the 84 genes on the SA Bioscience Breast Cancer and Estrogen Signalling Array results for MCF-7 cells treated with the controls and pesticides indicated. Two plots are presented with different Y axis scales to show high induction genes (more than 10 fold) and other genes induced or repressed. Results shown are average induction values from at least three separate cell treatment experiments normalized to the no treatment blank induction level. Replicate treatment experiment means shown are derived from multiple experiments that produced gene induction response reproducibility within the 0.01 p-value cutoff as determined by the SA Bioscience analysis spreadsheet. Typical estrogen responses are shown in the E2 (estradiol) treatment which may be considered the internal positive control. Complete analysis of these results is underway to determine which genes induced by pesticide mixtures should be focused on in Aim 3.





Highlights from this data set include:

1. E2 induced an repressed the genes expected for these cells.
2. Organophosphate pesticides did not induce estrogen dependant genes.
3. In binary mixture, methyl parathion potentiates the activity of opDDT and HPTE
4. In binary mixture, parathion potentiates the activity of ppDDT, HPTE, Bisphenol A
5. While E2 down regulates the GABA receptor, mixtures including parathion or fenitrothion potentiated or rescued this down regulation: opDDT, ppDDT, HPTE, Bisphenol A

Aim 3: Confirm the expression pattern of genes identified by microarray through analysis of gene products (mRNA or protein).

(a) Select 6−10 genes that have been shown by differential display to have novel expression patterns as a result of pesticide mixture treatment (Months 14−36).

(b) Obtain probes for Northern blot analysis of selected genes (Months 14−36).

(c) Perform Northern blots to confirm expression observed in micro array studies (Months 24−48).

(d) Obtain antibodies for Western blot analysis of selected genes (Months 14−36).

(e) Perform Western blots to confirm expression observed in micro array studies (Months 24−48).

Year Five Progress (April 19, 2008-April 18, 2009)

With the completion of the PCR Arrays in Aim 2, we this also completes sections a and b of Aim 3. With the PCR arrays replacing the Gene Chip arrays in the original Aim 2, the arrays perform the screen function and also the Real Tim PCR analysis function at the same time. A complete gene by gene analysis is underway to move on to Aim 3 sections c-e.

No Cost Extension Goals:
1. Complete Aim 3 sections c-e
2. Prepare and submit a manuscript describing this study and results.
3. Prepare a collaborative research proposal utilizing this study as preliminary data.

Era of Hope 2008 Abstract:

Interactions of estrogenic pesticides on breast cancer cell gene expression evaluated with a cancer focused PCR Array

Thomas E. Wiese[1], Huiming Li[1], H. Chris Segar[1], Steven R. Hill[2]
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A number of pesticides have been shown to stimulate estrogen receptor (ER) mediated proliferation and induce gene expression in breast cancer cells in culture. Thus, exposure to such compounds could be a contributing factor in the progression of breast cancer. We have previously described the estrogen regulated proliferation and reporter gene activity of DDT isomers and metabolites as well as of methoxychlor and it's primary metabolite HPTE. In addition, we have shown that some organophosphate pesticides may potentate this estrogen activity in particular binary mixtures. The goal of this study is to characterize the interactive effects of binary mixtures of pesticides and metabolites with estrogen activity on breast cancer cell gene induction. Most published characterizations of the hormone activity of pesticides in breast cancer cells have measured only the effects of single, pure compounds. Considering that real life exposure includes multiple isomers and/or the production of metabolites, it is of interest to examine what effect a mixture of these isomers and metabolites may have on genes related to breast cancer etiology and progression. MCF-7 cells were treated with mixtures of estradiol and one of the test pesticides as well as various mixtures of two pesticides, including combinations of one DDT and one of the organophosphate pesticides fenitrothion, methylparathion or parathion. RNA collected from these cultures was then used in Breast Cancer and Estrogen Receptor Signaling PCR Arrays from SuperArray. Analysis of these arrays in relation to each other and arrays from controls (blank, estradiol, single pesticides) are presented with highlight of novel gene expression patterns resulting from binary pesticide mixtures.

Deliverables/measurable outcomes:
Drs. Wang and Wiese will prepare or oversee the following:

1. Semiannual reports will be submitted to the PI.

Year Five Progress (April 19, 2008-April 18, 2009)
These reports were submitted and have bee used to make this progress report.

2. Students involved in the research will present a poster at the annual research workshop (Months 12, 24, 36, 48).

Year Five Progress (April 19, 2008-April 18, 2009)

Drs. Wiese and Wang have had no students working on their projects in Y5. Dr. Wolfgang (see Task 2a below) has had two students working with him on his new project: Thy Ho-Pham, Vi Tran. Dr. Muniruzzaman has had one student, Candace Hopgood working with him on his project. It should be noted that all Xavier students involved in the DOD programs at Xavier present at the universities annual Festival of Scholars in April.

3. One competitive grant application will be submitted by the end of the funding period.

Year Five Progress (April 19, 2008-April 18, 2009)

"MEK5-Erk5 pathways in survival signaling and tumor progression to drug resistance"
(**Principal Investigator**) 25% effort
Agency: NIH-NCI (BMCT), Type: 1**R01CA**125806-01A1, 07/01/09-06/30/14, $1,856,250
The long-term goal of this project is to identify the signaling pathways critical to the development of resistance to chemotherapeutics agents and the progression to a hormone independent phenotype in carcinoma of the breast.
Submitted July 2008 - **(scored 219)**

A proposal for the DoD IDEA AWARD (oppW81XWH-08-BCRP-IDEA)was submitted in May, 2008 and not funded. The title: MEK5-Erk5 Signaling Regulates Epithelial-to-mesenchymal Transition in Breast Cancer Progression.

A proposal was submitted to NIH titled "Proteomic and Phosphoproteomic Study of MEK5-Erk5 Signaling and Rapid Estrogen Signaling in Breast Cancer Cells". The proposal was submitted to NIH's SCORE program as an SC-1 type on January 25, 2009.

As the proposed Program Director, Dr. Guangdi Wang prepared and submitted an institutional program proposal entitled "Xavier's RCMI Cancer Research Program" to NIH's NCRR division on January 25, 2009.

4. Papers will be submitted to peer reviewed journals through the funding period.

Year Five Progress (April 19, 2008-April 18, 2009)
A manuscript has been published by Project #1 *Breast Cancer Research* in Dec 2008:

Changhua Zhou, Ashley M Nitschke, Wei Xiong, Qiang Zhang, Yan Tang, Michael Bloch, Steven Elliott, Yun Zhu, Lindsey Bazzone, David Yu, Christopher B Weldon, John A McLachlan, Rachel Schiff, Barbara S Beckman, Thomas Wiese, Kenneth P Nephew, Bin Shan, Matthew Burow and Guangdi Wang; **Proteomic analysis of tumor necrosis factor-alpha resistant human breast cancer cells reveals a MEK5/Erk5-mediated epithelial-mesenchymal transition phenotype. *Breast Cancer Research*** 2008**;10(6):R105. Epub 2008 Dec 16.**

Training deliverables:

1. The Tulane Cancer Center in conjunction with the Section of Hematology and Medical Oncology and The Cell Signaling group will be directly involved in providing breast cancer research training for Xavier Investigators.

Year Five Progress (April 19, 2008-April 18, 2009)

The support provided from the TCC to each project is described within the progress reports of each project above. In addition, TCC support for the program as a whole is detailed in Task 3 below.

2. Toward the end of the project period, Drs. Wang and Wiese will be Co−PIs in writing an R01 grant in collaboration with Drs. Burow and Hill.

Year Five Progress (April 19, 2008-April 18, 2009)
While no R01 collaborative grants are in preparation at this time, Dr. Burow (Tulane) from Project #1 will submitt an R01 grant related to this project and, Project #1 team (Drs. Wang and Burow) will submit a DOD Idea award in May 2008 (see Aim 3, Part 3 above).

Task 2

Assist two Xavier junior faculty to become more competitive in breast cancer research

a. Identify two Junior Faculty with interest in breast cancer research (Month 1).

Year Five Progress (April 19, 2008-April 18, 2009)
Dr. David Wolfgang, XU Chemistry, has developed a project with Dr. Charles Miller of the TCC (see below). Dr. Syed Muniruzzaman, XU Biology, has developed a project with Dr. Wiese, XU Pharmacy, involving the evaluation of rare sugars as potential cancer chemotherapeutic agents (see below).

b. Establish participation of the selected Junior Faculty in Tulane Cancer Center seminars and the weekly signal transduction workshop focused on breast and prostate cancer (Month 2).

Year Five Progress (April 19, 2008-April 18, 2009)
All faculty involved in the Xavier DOD Cancer programs are now integrated into the Molecular Signaling focal group of the LCRC. These faculty are exposed to a wide range of cancer research by attending the Molecular Signaling research meetings and these faculty are now building stronger ties between Xavier and the LCRC. We have established a twice monthly Cancer Research lunch meeting at Xavier (see below).

c. Determine Tulane Cancer Center mentors for the Junior Faculty and submit a two−page mini proposal for review of the PI and alternate PI (Month 6).

Junior Faculty Project #1
Dr. David Wolfgang (XU Chemistry) has developed a project with Dr. Miller at the TCC and progress is reported below.

Note: In Fall 2007, Dr. Wolfgang was not approved for tenure at Xavier. He accepted a terminal contract ending May 2009. Considering that he has made significant progress on his project and that he continues to involve Xavier students in his research, The PI elected to continue supporting Dr. Wolfgang in this program through the spring of 2009.

The Effect of Cellular Levels of the Hsp90 Co-chaperon p23 on the Stress Response of Mice Fibroblasts.

Original Specific Aims:

1) To determine if the amount of p23 in mouse fibroblasts affects the toxicity of anti-tumor compounds geldanamycin and herbimycin A.

2) To determine if the amount of p23 in mouse fibroblasts affects the toxicity of compounds (cadmium and arsenate) known to initiate the heat shock response.

New Specific Aim:
To produce a peptide that will bind to p23 and inhibit the binding of p23 to Hsp90, making the cells functionally p23 null.

Introduction:
Hsp90 is a chaperon protein that plays a role in the maintenance of steroid hormone receptors in their high affinity form. Hsp90 also interacts with kinases and polymerases. Proper Hsp90 function requires additional factors (co-chaperones) such as p23. Hsp90 has been linked to proteins involved in all six features found in almost all cancers: 1) self sufficient growth signaling, 2) insensitive to signals that halt the cell cycle, 3) evade apoptosis, 4) angiogenesis, 5) metastasis, and 6) unlimited potential for cell division. As such, the understanding of Hsp90 and its co-chaperones is vital to understanding and treating cancer. The protein p23 has been shown to maintain Hsp90 in its active (ATP bound) form. p23 is also part of the Hsp90 complex that is involved in chaperoning estrogen receptor alpha. The fact that p23 is up-regulated in cancer cells suggests that it may play a role in tumor growth. It has also recently been shown that overexpression of p23 in MCF-7 cells enhances adhesion and invasion. Dr. Charles Miller has mice that are heterozygous for the p23 gene, one copy has been knocked-out. We obtained cells from embryos since mice that are p23 homozygous null are not viable beyond birth. After determining whether or not the presence of p23 plays a role in tumor growth, a peptide will be synthesized to hopefully disrupt the binding of the Hsp90-p23 chaperone complex.

Methods:
Mice heterozygous for the p23 gene are mated and females are sacrificed about a day before birth. Skin cells are plated in T25 flasks with DMEM media supplemented with 10% Fetal Bovune Serum, L-glutamine, sodium pyruvate, and antibiotics. When the plates are confluent, the cells are treated with trypsin to remove them from the flask, counted, diluted to 12,000 cells per mL, and seeded in 96-well plates at a concentration of 1200 cells per well. These cells are grown overnight at 37ºC. Doses of the test compound are diluted in DMEM media supplemented as described. Geldanamycin and Herbimycin are tested in the range of 10 μM to 1 nM final concentration. Cadmium and arsenate were tested in the range of 0.3 to 10 μM final concentration. The old media is suctioned out of the wells and replaced with 100 μL of media with the appropriate dose. Three sets of controls and prepared; wells with cells and "dosed" with media only function as a positive control, wells without any cells function as a negative control, and cells "dosed" with media and DMSO control for the fact that Geldanamycin and Herbimycin A are diluted from a stock solution dissolved in DMSO. The dosed cells are returned to 37ºC and incubated for 24 hours, after which the dose is suctioned off and replaced with media. The plates are then returned to 37ºC for three days. At the end of the three days 10 μL of 0.1% (w/v) alamar blue (resazurin) diluted in phosphate buffered saline is added. The plates are returned to

37ºC to allow the surviving cells to reduce the alamar blue into resorufin. The resorufin fluoresces at 590 nm and this fluorescence is measured usually at 6-8 hours after addition of alamar blue. The fluorescence from the positive control is set at 100% and the fluorescence from the negative control is set at 0 %. The data is fit to a sigmoidal dose response curve and a concentration that yields 50% fluorescence is expressed as the EC50 value.

Results:
As of the last report, one litter of mice had been tested. Two additional litters have been tested. The actual results are shown in Table 1. For each compound tested the TD50 value decreases from wild-type to heterozygous to null. However due to high standard deviations the only statistically significant difference is between WT and null for arsenate (p=0.05).

Table 1. TD_{50} values for Herbimycin A, Cadmium, Geldanamycin, and Arsenate tested on primary mouse embryonic fibroblasts.

Compound	WT	Heterozygous	Null
Herbimycin A nM	676 +/- 594 (n=7)	376 +/- 341 (n=8)	180 +/- 134 (n=6)
$CdCl_2$ µM	4.3 +/- 1.7 (n=7)	4.2 +/- 2.14 (n=8)	3.39 +/- 0.68 (n=6)
Geldanamycin nM	11.1 +/- 5.4 (n=5)	8.11 +/- 2.44 (n=8)	6.16 +/- 3.2 (n=5)
Arsenate µM	199.6 +/- 87 (n=4)	159.9 +/- 97.2 (n=7)	97.5 +/- 32.6 (n=5)

Because of high variability in primary mouse embryonic fibroblasts (MEFs) the data was normalized as follows. For a given litter the average TD50 was calculated regardless of genotype. This average was given a value of 1.00. All values were expressed relative to this average. For example if the average TD50 value for geldanamycin was 10 nM and a null embryo had a TD50 value of 4.5 nM this embryo was listed as having a TD50 of 0.45. The purpose was to remove variability between litters. In the case of each compound tested the TD50 values for null MEFs were below 1.00 and the TD50 values for both wt and heterozygous MEFs were above 1.00 (Table 2).

Table 2. Normalized TD_{50} values for Herbimycin A, Cadmium, Geldanamycin, and Arsenate tested on primary mouse embryonic fibroblasts.

Compound	WT	Heterozygous	Null
Herbimycin A nM	1.44 +/- 0.79 (n=7)	1.20 +/- 0.80 (n=8)	0.53 +/- 0.30 (n=6)
$CdCl_2$ µM	1.00 +/- 0.20 (n=7)	1.09 +/- 0.47 (n=8)	0.86 +/- 0.15 (n=6)
Geldanamycin nM	1.22 +/- 0.53 (n=5)	1.01 +/- 0.27 (n=8)	0.75 +/- 0.45 (n=5)
Arsenate µM	1.16 +/- 0.29 (n=4)	1.13 +/- 0.32 (n=7)	0.68 +/- 0.28 (n=5)

When the data is expressed in this normalized fashion there are more statistical differences (Table 3). Now Herbimycin and Geldanamycin show statistical differences. Cadmium was not significant regardless of how the data was analyzed.

Table 3. Student t-test t values. Cut off values for p = 0.05 are shown in parentheses.

Compound	Normalized WT vs. Null	Normalized WT & HZ vs. Null
Herbimycin A	2.606* (2.179)	2.39* (2.086)
CdCl2	1.659 (2.179)	1.321 (2.086)
Geldanamycin	2.33* (2.262)	1.62 (2.120)
Arsenate	2.40* (2.262)	3.043* (2.120)

From the data, it was determined that cells null for p23 were more susceptible to drugs. With this knowledge in hand, the MOE (molecular organizational environment) program was used to visualize the binding of the mouse Hsp90 homolog (Hsp82) to the mouse homolog of p23 (SBA1) in the attempt to create a peptide that would bind to the p23 and inhibit p23-Hsp90 binding. The crystal structure for Hsp82-SBA1 chaperone complex has been solved ***reference***. This chaperone complex was analyzed and sites of best interaction were isolated based on various computations using the docking feature of MOE. The MOE program had not been previously used; therefore, it took some time to learn the different features of the program and even more time to teach the students involved in the project how to correctly use MOE. From the paper by Ali et al., it was known which parts of Hsp82 interacted with SBA1. The MOE program helped us visualize these interactions sites, and it was found that SBA1 had a concave region that interacted with Hsp82. It was then theorized that the concave region might function as a receptor to a small peptide. Using the MOE program, a small peptide was made whose sequence corresponded to the Hsp82 sequence which binded to the concave region of SBA1. Using the docking feature of MOE, it was found that the peptide interacted with the predicted concave region of SBA1. To ensure that the concave region was a specific binding site, a random peptide was generated and docked with SBA1. Unfortunately, this peptide interacted with SBA1; however, the interaction occurred at a different site. It was anticipated that the region involved in binding would be conserved among various species. A multiple alignment of sequences was performed with Hsp90 or its homolog from ten various organisms (Fig 1). The proposed sequence for the peptide is highlighted below:

Fig. 1: Multiple alignment sequences of ten organisms

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FOSB_HUMAN            IRYESLTDPSKLDSGKELHINLIPNKQDRTLTIVDTGIGMTKADLINNLGTIAKSGTKAF 240
FOSB_MOUSE            IRYESLTDPSKLDSGKELHINLIPSKQDRTLTIVDTGIGMTKADLINNLGTIAKSGTKAF 118
FOSB_CHICKEN          IRYESLTDPSKLDSGKDLKINLIPNKHDRTLTIVDTGIGMTKADLVNNLGTIAKSGTKAF 117
FOSB_ZEBRAFISH        IRYESLTDPSKLDSCKDLKIELIPDQKERTLTIIDTGIGMTKADLINNLGTIAKSGTKAF 115
FOSB_BOVINE           IRYESLTDPSKLDSGKELKIDIIPNPQERTLTLVDTGIGMTKADLVNNLGTIAKSGTKAF 113
FOSB_FROG             IRYESLTDPSKLDSGKDLKIDIIPNRLERTLTMIDTGIGMTKADLINNLGTIAKSGTKAF 113
FOSB_DROSOPHILA       IRYESLTDPSKLDSGKELYIKLIPNKTAGTLTIIDTGIGMTKSDLVNNLGTIAKSGTKAF 106
FOSB_FLUKE            IRYKSLTEPSVLDTESELCIKVIPNKADSTLTIIDTGIGMTKADLVNNLGTIARSGTKAF 116
FOSB_ARABISOPSIS      IRFESLTDKSKLDGQPELFIHIIPDKTNNTLTIIDSGIGMTKADLVKNLGTIARSGTKEF 105
FOSB_YEAST            IRYKSLSDPKQLETEPDLFIRITPKPEQKVLEIRDSGIGMTKAELINNLGTIAKSGTKAF 104
                      **::**:: . *:   :* * : *.    .* : *:******::*::******:**** *

FOSB_HUMAN            MEALQAGADISMIGQFGVGFYSAYLVAEKVTVITKHNDDEQYAWESSAGGSFTVRTDT-G 299
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FOSB_MOUSE            MEALQAGADISMIGQFGVGFYSAYLVAEKVTVITKHNDDEQYAWESSAGGSFTVRTDT-G 177
FOSB_CHICKEN          MEALQAGADISMIGQFGVGSYSAYLVAEKVTVITKHNDDEQYAWESSAGGSFTVRLDN-G 176
FOSB_ZEBRAFISH        MEALQAGADISMIGQFGVGFYSAYLVAEKVTVITKHNDDEQYIWESAAGGSFTVKPDF-G 174
FOSB_BOVINE           MEALQAGADISMIGQFGVGFYSAYLVAEKVVVITKHNDDEQYAWESSAGGSFTVRADH-G 172
FOSB_FROG             MEALQAGADISMIGQFGVGFYSAYLVAEKVVVITKHNDDEQYAWESSAGGSFTVKVDT-G 172
FOSB_DROSOPHILA       MEALQAGADISMIGQFGVGFYSAYLVADKVTVTSKNNDDEQYIWESSAGGSFTVRADN-S 165
FOSB_FLUKE            MEALNAGADISMIGQFGVGFYSAYLVADKVQVISKNNDDEQYLWESSAGGSFTIRPCS-E 175
FOSB_ARABISOPSIS      MEALAAGADVSMIGQFGVGFYSAYLVADKVVVTTKHNDDEQYVWESQAGGSFTVTRDTSG 165
FOSB_YEAST            MEALSAGADVSMIGQFGVGFYSLFLVADRVQVISKSNDDEQYIWESNAGGSFTVTLDEVN 164
                      **** ****:********* ** :***::* * :* ****** *** ******:
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As shown, the proposed sequence is almost identical in all organisms with the exception of two amino acids. This supports the idea that this region is important in binding. The peptide will be synthesized using the highlighted sequence from the Hsp82: FMEALSAGADV.

After the sequence for the peptide was determined,

The peptide will be synthesized as a fusion protein. Using the pET42 vector from Novagen the DNA sequence coding the peptide will be cloned into the vector using restriction enzymes PshAI and NcoI. This will allow the expression of a fusion protein (peptide fused to GST Tag) that can be purified with a glutathione column. The peptide will then be released from the Vector sequence using factor X_a.

The assay for binding will carried out as outlined (Cox and Miller). SBA1 with a 6-His tag will be expressed in E. coli, purified on a His-Bind resin, and then dialyzed to remove imidizole. Wild-type Hsp82 will also be expressed in E. coli. An aliquot of Hsp82 cell lysate will be incubated with purified SBA1in the presence of ATP-γ-S. This mixture will be added to His-Bind resin, incubated, washed and pelleted. The pellet will contain the His-Bind resin and any proteins that bind to it, either directly (SBA1) or indirectly (Hsp82). The pellet will be boiled with SDS-PAGE sample buffer, and the supernatant loaded onto SDS PAGE gels. Appearance of Hsp82 on the gel will be indicative of SBA1-Hsp82 binding. If the peptide is added to the mixture and interferes with SBA1-Hsp82 binding, then a lower amount of Hsp82 will be seen on the gel. Mutant Hsp82 will be used as a negative control for the binding.

Vectors containing SBA1 with a 6-his tag, wild-type Hsp82, and mutant Hsp82 were provided by Charles Miller. We have worked out conditions for the expression and partial purification of SBA1. We have the Hsp82 lysate, ran a SDS page of the lysate but saw no difference between induced and uninduced Hsp82. However, even if the protein is expressed, one cannot always see a difference on the gel. Therefore, we cannot be certain that we have expression of Hsp82. ATP-γ-S is on order from Sigma and when it arrives the assay will be run to determine if there is binding between Hsp82 and SBA1. If no binding is observed, the EnzChek phosphate assay kit for Molecular Probes will be used to determine if Hsp82 is expressed.

Year Five Progress (April 19, 2008-April 18, 2009)

Poster presentation at Annual Festival of Scholars, April 2009, Xavier University

Design of a Peptide to Disrupt the Binding Interaction of Hsp90 and p23.
David E. Wolfgang, Vi Tran, Thy Ho-Pham, and Charles Miller*
Department of Chemistry, Xavier University of Louisiana, New Orleans 70125
*Department of Environmental Health Sciences, Tulane University, New Orleans 70112

Hsp90 is a heat shock protein that helps proteins re-fold under stressful conditions, such as cancer. P23 is a co-chaperone of Hsp90. P23 binds to the dimer form of Hsp90 and enhances its

activity. In certain cancers, an elevated expression of Hsp90 and p23 helps mutated proteins retain their proper conformation, allowing for rapid cell growth. This complex is a potential target for the treatment of cancer. Preliminary results indicate that mouse embryonic fibroblast cells null for p23 are more susceptible than wild-type cells to treatment with Hsp90 inhibitors such as geldanamycin and herbimycin. By interfering with the interaction of Hsp90 and p23, the cells should be functionally p23 null. It is anticipated that these "functionally p23 null" cells will exhibit an increased sensitivity to geldanamycin and herbimycin. The x-ray crystal structure of the yeast p23 and Hsp90 complex is known. Based on this structure a rationally designed peptide will be produced to interfere with the Hsp90-p23 interaction. The peptide will then be screened using a computer model. Peptides that show tight binding on the computer model will be tested with an in-vitro binding assay.

Junior Faculty Project #2

Dr. Syed Muniruzzaman, Xavier Biology, has developed a project with Thomas Wiese, Xavier Pharmacy.

Evaluation of Rare Carbohydrates as Inhibitors of Various Biological Processes

On the basis of availability we can classify monosaccharide into two groups, natural and rare. Rare carbohydrates are not abundant in the nature and difficult to produce by organic or chemical reactions. The practical application or usefulness of rare carbohydrates has not been well investigated because of high costs and unavailability. Despite their costs, these rare carbohydrates are very important since they have the potential for use in many areas. For instance, a rare keto hexose, D-tagatose, is now attracting much attention as a low-calorie carbohydrate sweetener and bulking agent (1, 2). D-psicose, a rare keto hexose, does not provide any energy and has other beneficial clinical effects when given to animal orally (3, 4). Recently, it has been reported that a rare aldohexose, D-allose, substantially inhibits segmented neutrophil production and lowers platelet counts without any detrimental clinical effect, might be used in the treatment of myeloid leukemia (5, 6). Another study reported that D-allose inhibited human ovarian carcinoma cells in vitro (7). In February of 2008 Yamaguchi et al reported that D-allose significantly up-regulated thioredoxin interacting protein (TXNIP) gene expression and subsequent G1 cell cycle arrest in hepatocellular carcinoma cells by stabilization of p27kip1(8).

In a previous study Muniruzzaman et al found that rare ketoses, L-fructose and L-xylulose are potential inhibitors of glycoprotein processing enzyme in cell culture system (9). Also, the use of some derivatives of rare carbohydrates as a potent antiviral agent against hepatitis B virus and human immunodeficiency virus have been reported (10, 11). Some other derivatives are also reported as anti-tumor agent for example, bleomysin, which is active against several murine tumors thereby making it useful for cancer treatment (12). Rare sugars are also important as the building block for the synthesis of L-oligo nucleotides and enantio DNA (DNA having L sugar), which are valuable tools for studying protein DNA interactions and are promising antisense agents (13). Utilization of rare sugars for various other purposes still awaits exploration.

The goal of this study is to explore the effects of rare hexoses, pentoses and their deoxy-derivatives on the cancer cell lines. However, initially this study would like to see the effect of only rare ketohexoses on breast cancer cell lines. Amongst the eight ketohexoses L-Fructose, D-Psicose, L-Psicose, D-Sorbose, D-Tagatose and L-Tagatose are rare. These rare ketohexoses will be studied in appropriate cell lines to see any inhibitory or cytotoxic effect.

The effects of the rare sugars will be examined using cell culture bioassays that are routinely used in the Wiese lab that evaluate breast cancer cell proliferation/survival and estrogen reporter gene activity. Our hypothesis is that some of the rare sugars will reduce breast cancer cell proliferation and/or estrogen reporter gene activity with low, general cytotoxicity.

Year Five Progress (April 19, 2008-April 18, 2009)

Abstract presented at Annual LCRC Retreat, March 2009

Rare Ketohexoses as Inhibitors of Breast Cancer Cell Proliferation and Estrogen Signalling

Candace Hopgood[1], Thomas E. Wiese[2], S P Kale[1], Syed Muniruzzaman[1]
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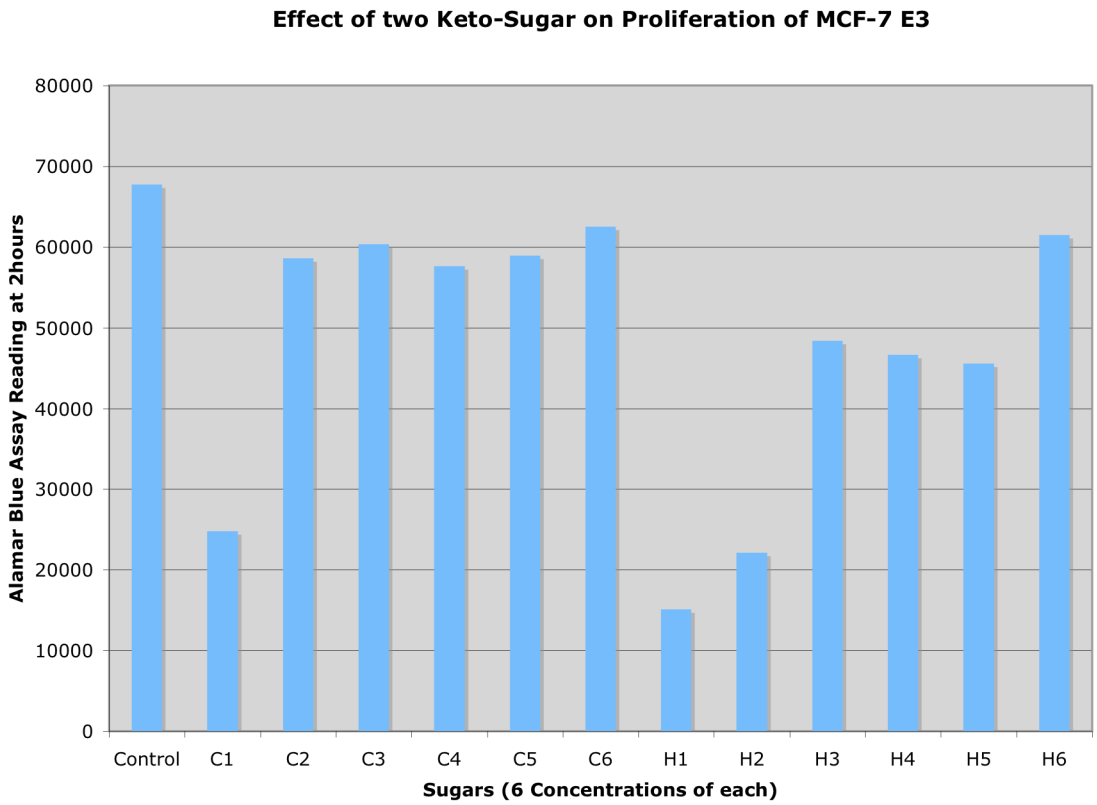
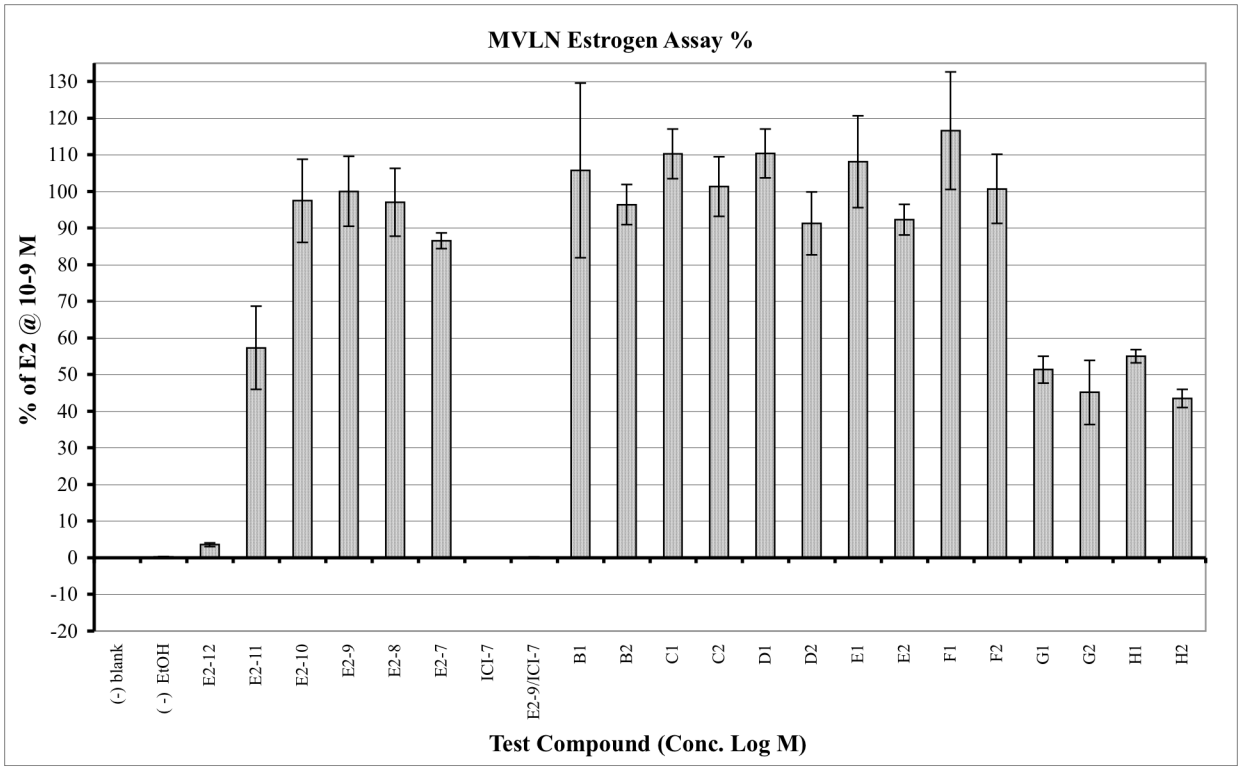
[2]Xavier University of Louisiana College of Pharmacy, New Orleans, LA, 70125

Rare carbohydrates are not abundant in the nature and difficult to produce by organic or chemical reactions. On the basis of availability we can classify monosaccharides into two groups, natural and rare. Amongst the eight ketohexoses L-Fructose, D-Psicose, L-Psicose, D-Sorbose, D-Tagatose and L-Tagatose are rare. In this study we have determined the effect of these rare ketohexoses on breast cancer cell proliferation and estrogen signalling. The Alamar Blue assay was used to determine the effect of the sugars on estrogen induced proliferation in a 96 well plate format. These same sugars were evaluated for effect on estrogen regulated transcription in MVLN cells (MCF-7 stably transfected with Vit-Luc-Neo) in 96 well plate format. Among these rare ketoses, two showed inhibitory effect on estrogen at mM concentration.





No Cost Extension Goals:

1. Continue characterizing the antiestrogenic and antiproliferative properties of the rare sugars.

2. Prepare and submit a manuscript describing this study and results.
3. Prepare a collaborative research proposal utilizing this study as preliminary data.

d. Junior Faculty collect preliminary data (Months 7−36).

Year Five Progress (April 19, 2008-April 18, 2009)
See Task 2 c for current status of the Wolfgang-Miller and Muniruzzaman-Wiese projects.

e. Junior Faculty develop grant proposal (Months 36−48).

Year Five Progress (April 19, 2008-April 18, 2009)
No Progress in this area in Y5.

Task 3
Establish infrastructure that will create an environment that fosters breast cancer research, in which Xavier faculty will receive substantive training and become more competitive for DoD funding

Background and Year Five Progress (April 19, 2008-April 18, 2009)
When Xavier was awarded the DOD Breast Cancer grant in April 2004, Dr. Rosenzweig, the project PI, announced that she would leave Xavier in May 2004. A plan was formulated where Dr. Wiese, PI of one of the research projects in the Breast Cancer training program would take over program PI responsibilities along with his research project. He would be provided release time for both tasks and be assisted by a part time administrative assistant that would be hired. Dr Wiese served 5 years as a joint faculty between Tulane and Xavier before moving full time to Xavier in 2003. While at Tulane, he became a member of the Tulane Cancer Center and developed a good working relationship with Dr. Steven Hill, Tulane coPI of this project. Dr. Wiese also had also developed a good working relationship with Dr. Klassen, Xavier Chemistry Department, coPI of the XU-YU DOD Prostate Cancer training program, when Dr. Klassen utilized the cell culture facilities in the Wiese lab in 2003-2004.

Unfortunately, Dr. Klassen elected not to return to Xavier after Katrina. Dr. Wiese, the PI of the Xavier DOD BC program has been asked by the Xavier administration to replace Dr. Klassen as PI of the Xavier DOD Prostate program effective Feb 2006.

The Xavier DOD BC and PC programs continue to operate in parallel with meetings, seminars and discussion sessions involving both groups. In Y1, we established an email list serve for all Xavier and Tulane faculty involved in both XU DOD cancer training projects and this mechanism has been very helpful for rapid communication of cancer center events, project meetings and organizing car pools to LCRC seminars.

It should be noted that the Tulane Cancer Center is part of the Louisiana Cancer Research Consortium (LCRC) that includes the LSU Cancer Center. The LCRC was devised in 2002, involves significant funding from the state of Louisiana and will eventually be housed in a new building between the Tulane and LSU medical centers in New Orleans. The LCRC is co-directed by Dr. Prescott Deininger (Director of Tulane Cancer Center) and Dr. Augusto Achoa (Director of the LSU Cancer Center). Drs. Klassen and Wiese were invited to the first annual

LCRC retreat in January 05. The planning process and meetings that took place at this retreat clearly stated that all Xavier faculty interested in or doing cancer research were welcome to participate in the LCRC through adjunct appointments in Tulane or LSU departments. In addition, Dr. Roy Weiner (previous TCC director) has keep in close contact with Dr. Wiese regarding the Xavier DOD Breast Cancer training program and has made it clear that he is personally committed to helping Xavier faculty develop cancer research projects and programs. He has opened up all the resources of the Tulane Cancer Center core facilities to Xavier researchers and has invited Xavier faculty to be involved in the Tulane Cancer Centers cancer research symposia held each fall. This Mauvernay Research Excellence Award program includes seminars and posters related to cancer research and concludes with a dinner where TCC faculty meet the invited speakers. Several of the XU faculty involved in the DOD cancer training programs attended the Mauvernay Research Excellence Award program in fall 2006 and Drs. Hill and Weiner made a special effort to introduce the XU faculty to TCC faculty and to the invited speakers. Dr. Weiner also has included clinical faculty from the Xavier College of Pharmacy in ongoing initiatives at the Tulane Cancer Center.

One result of this close relationship between Drs. Weiner and Hill of the TCC and Xavier University is the submission of a joint P20 planning grant to the NCI in February of 2005 with Xavier University of Louisina. This grant was specifically designed to plan long term collaborations between cancer centers and minority serving institutions. Through a series of meetings starting in October 2004, a P20 grant was developed between the Tulane Cancer Center and Xavier University with Dr. Weiner as the Tulane PI and Dr. Kathleen Kennedy, Associate Dean, Xavier College of Pharmacy as the Xavier PI. At the same time, the PI and co-PI of the Xavier DOD Breast Cancer Training Program, Drs. Wiese and Hill became the P20 grant program managers for each respective institution. Drs Wiese and Hill also took responsibility for the majority of the organization, planning and preparation of this panning grant over a 5 month period leading up to submission in February 2005. This NCI P20 program grant was awarded in August 2005 with a start date of October 1, 2005 (during the Katrina evacuation). The good working relationship of Dr. Wiese and Hill, developed largely from the DOD Breast Cancer Training Program and other prior activities, was critical to working out the complex details of this P20 proposal that involved two very different universities. **We feel that the DOD cancer training programs between Tulane and Xavier provided the critical mass required to put together this P20 grant and that the combination of these programs has contributed significantly to the development of self sustaining cancer research programs at Xavier: Xavier as member of the Louisiana Cancer Research Consortium (LCRC) and new RCMI grant submitted to support cancer research (see Task 1, Project 1 above and following description of XU LCRC involvement).**

The review of the original Xavier DOD Breast Cancer Training program requested that an administrative assistant be hired to assist the PI in grant management tasks as well as in planning meetings and coordinating communication between all those involved at XU and TU. In August 2004, Mr. Sergio Alcantera was hired as a part time program manager for this project. See Y1 progress report for more details. Mr. Alcantera moved his family to California after Katrina leaving this position open.

With the award of the NCI P20 training grant in 2005, Xavier now had two program grants that had openings a program assistant. With the help of Dr. Roy Weiner at the Tulane Cancer Center and a search process at Xavier, a sutable candidate was identified in early 2006. Ms. Stephanie Colbert was hired by Xavier in February 2006 to support both the DOD BC program and the

NCI P20 grant working under the supervision of Dr. Wiese, PI of the DOD BC program and manager of the NCI P20 program.

In the fall of 2007, Xavier University of Louisiana was brought into the Louisiana Cancer Research Consortium (LCRC) to joint Tulane and LSU in the development of a Louisiana Cancer Center that will eventually achieve NCI designation. The LCRC was established in 2002 with state tobacco tax funds and it supports ~$10,000,000 of cancer research and smoking cessation activates each year. While all cancer researchers at Xavier had been members of the LCRC (through involvement in DOD programs and membership in the Tulane Cancer Center), this new status brings LCRC resources directly to Xavier. In the 2007-2008 state fiscal year, Xavier received $500,000 to develop LCRC activities and starting in the 2008-2009 fiscal year, Xavier will receive $900,000 each year to develop and maintain cancer research programs. **It is important to recognize that Xavier involvement in the LCRC is directly related to Xavier involvement in the DOD Cancer Training programs.**

The logical choice for the leadership for the Xavier LCRC participation was Xavier faculty already involved in leading cancer programs funded by the DOD and NCI. Dr. Wiese was established as the LCRC Associate Director for Xavier and Ms. Colbert as the Xavier LCRC program assistant. Dr. Wiese now attends twice monthly LCRC leadership meetings with the co-Directors, CEO and Administrator for Research as well as the every other month LCRC board meetings and Finance meetings. He also is a member of the LCRC Scientific Executive Committee that meets monthly to deal with review of LCRC supported internal funding requests, review and development of core facility operations and LCRC long term planning. Ms. Colbert attends the LCRC business manager, finance and board meetings in addition to working with the LCRC staff on public relations, annual reports, news letters and some fund raising organization.

The first task of the Xavier LCRC was to plan goals and structure at Xavier. An internal advisory board was established consisting of the chairs of the Biology, Chemistry, Division of Basic Pharmaceutical Sciences, Division of Clinical and Administrative Sciences, the Director of the Xavier Center for Health Disparities Research and Education and the Senior VP for Resource Development. This XU LCRC IAB meets every two months to advise and over see the activities of the XU LCRC Associate Director. In addition, the IAB reviews submissions requests for XU LCRC funding (pilot grants, seed grants, instrument requests, etc) and serves as a direct line of communication to and from university units with the bulk of the cancer researchers on campus. While XU LCRC IAB represented units are looking to recruit faculty, the XU LCRC will with them to hire and support cancer research focused faculty.

A plan was developed where the bulk of the XU LCRC funding would go to new hire start up packages (cancer research only) while cancer research core infrastructure, pilot and seed grants, development of core facilities and cancer research seminars and research discussion meetings would also be supported.

In 2007-2008, some cancer research faculty just hired were supported with small startups. In 2008-2009, a coordinated recruitment resulted in the hire of 6 cancer research focused faculty that are now receiving three years of startup funding to establish their research at Xavier. In the fall of 2008, Xavier LCRC Associate Director Dr. Wiese submitted a proposal to the LCRC for an additional $1.6 million for the sole purpose of continuing the recruitment of cancer research faculty. With the regular Xavier LCRC budget largely committed to support startup packages for recent hires, this supplement provides funds to hire at least 4 more faculty doing cancer

research. Searches in Chemistry, Biology and Pharmacy are underway.

Xavier involvement in the LCRC has provided the ability to recruit cancer research focused faculty (start ups) and to sustain cancer research activities developed by the XU DOD Cancer training programs (seminars, research discussion meetings, development of new projects, research infrastructure).

The XU LCRC budget for FY2010 as well as the Xavier LCRC supplemental budget are included in Appendix 1

With Xavier a member of the LCRC, Dr. Wiese the Associate LCRC Director for Xavier, and Xavier President Dr. Norman Francis a member of the LCRC board, Dr. Wiese is in close contact with Dr. Francis as well as senior LCRC administrators about the development of cancer programs at Xavier.

a. Grant membership in the Tulane Cancer Center to Xavier researchers. Drs. Wang and Wiese will be granted a status of contributing members and the junior faculty will be granted a status of associate members. Please see attached TCC publication for the definitions (Month 1).

Year Five Progress (April 19, 2008-April 18, 2009)
All 4 Xavier faculty involved in this program have either been approved as adjunct faculty at Tulane or this approval is pending. Once approved, this status allows Xavier faculty to be contributing members of the Tulane Cancer Center (TCC) as well as the Louisiana Cancer Research Consortium (LCRC). As members, these faculty can use the various core facilities at the cancer center at a reduce rate. To date, all Tulane mentors have facilitated the use of any needed cancer center cores with or with out membership. This adjunct status also allows the Xavier faculty doing cancer research to use the Tulane library resources.

b. Include Xavier researchers in Tulane Breast Cancer focus group and Journal Club (Months 2).

Year Five Progress (April 19, 2008-April 18, 2009)
All faculty involved in the Xavier DOD Cancer programs are now integrated into the Molecular Signaling focal group of the LCRC. These faculty are exposed to a wide range of cancer research by attending the Molecular Signaling research meetings. In addition, these faculty are now involved in building stronger ties between Xavier and the LCRC. We have established a monthly Cancer Research lunch meeting at Xavier (see Task 3 e below).

It should be noted that Xavier cancer research faculty are informed about and encouraged to attend all the cancer related seminars and working groups in the LCRC. The LCRC invited speaker series is every other Thursday at noon alternating between Tulane and LSU. Other LCRC discussion groups include: Friday Afternoon Encounters to discuss recent data, the Immunology Club, the Apoptosis and Cell Survival meetings (2x per month) and the weekly Prostate Cancer Group Meetings at LSU; the weekly Breast/Ovarian Group Meeting and the weekly Stem Cells and Cancer Group Meeting at Tulane Cancer Center. The two DOD programs at Xavier have also established a bi-weekly Cancer research Discussion group where faculty involved in DOD projects rotate presenting about the latest status of their project. Tulane mentors and collaborators as well as students are invited to these meetings and this program has been very helpful in bringing our group together. This cancer focused work in progress series

has become very popular among faculty interested in cancer research and new faculty hired to do cancer research supported by the LCRC.

c. Grant access to core research facilities at Tulane Cancer Center (Month 1).

Year Five Progress (April 19, 2008-April 18, 2009)
Access to TCC and LCRC core facilities has been granted to Xavier faculty. These cores include: Genomics, Proteomics, Biostatistics/Bioinformatics, Immunology, and Tissue Acquisition. To date, no Xavier faculty have required the use of these facilities.

d. Include a student in each research project (Month 2 for Drs. Wang and Wiese and Month 8 for the junior faculty).

Year Five Progress (April 19, 2008-April 18, 2009)
See Task 1 above.

e. Establish a monthly brown−bag lunch meeting to bring up research related issues, review proposals and papers, or brainstorm on new directions to improve the cancer program (Month 1).

Year Five Progress (April 19, 2008-April 18, 2009)
We have established a monthly Cancer Research lunch meeting at Xavier where the faculty involved in our DOD Cancer programs rotate in giving "work in progress" presentations to the group. These meetings are held on a Monday at noon each month and are well attended by all members of the labs involved as well as our Tulane mentors-collaborators. These meetings have not only assisted Xavier faculty with their projects, but have also provided a place where we can all se what each other are doing. Other Xavier faculty interested in cancer research are now attending these meetings and we may expand these sessions to twice a month. The schedule for these meetings is listed in Appendix 2.

e. Hold an annual workshop, open to all in the Xavier and Tulane communities and Xavier student body, for all BC participants to present results of the preceding year. Faculty, students, and staff will attend and at least one person from each group will present a talk; students will present posters (Months 12, 24, 36, 48).

A. First workshop titled "Molecular Signaling in Breast Cancer" (Month 12).
B. Second workshop titled "Breast Cancer and the African American Community" (Month 24).
C. Third workshop titled "Funding Opportunities in Breast Cancer Research" (Month 36).
D. Forth workshop titled "Drug Design and Delivery in Breast Cancer" (Month 48).

Year Five Progress (April 19, 2008-April 18, 2009)
Our attempts to hold a Cancer Research symposia at Xavier have been foiled by scheduling conflicts with university and department events. We will continue trying to developed a seminar series at Xavier as well as a mini-symposia at with poster sessions. It should be noted that all Xavier students involves in the DOD programs at Xavier present at the universities annual Festival of Scholars in April. With Xavier now part of the Louisiana Cancer Research Consortium (LCRC), Xavier cancer research faculty will be presenting at the LCRC annual retreat held each spring. **Participation in this city wide symposia involving cancer**

researchers from Tulane and LSU along with Xavier achieves our goal of involving Xavier faculty in an annual cancer focused symposia. In 2009, this retreat was held at Xavier and plans are set for Xavier to hose the LCRC retreat in March of 2010.

f. Subscribe to breast cancer related journals (Month 1).

Year Five Progress (April 19, 2008-April 18, 2009)s

In Y1, we purchased a subscription to the online journal Breast Cancer Research. In Y2, we determined that we could get access to the journal Proteomics through the Xavier Library and that a subscription was not needed. Access to Tulane library resources is still limited for some XU faculty. Only faculty with adjunct appointments have off campus online access. Dr. Wiese has access (from prior adjunct appointment) and is serving as the access point for journal articles needed from Tulane. In Y3 we must established XU faculty as adjuncts at Tulane to resolve this problem. We now maintain these subscriptions and cancer research faculty at Xavier have access to the journals at the Tulane Library.

Key Research Accomplishments

- Comparative proteomics was carried out on the two breast cancer cell lines, MCF-7-MEK5 and MCF-7-VEC. As described earlier, MCF-7-MEK5 is a TNF-α-resistant breast cancer cell line derived from the wild-type, i.e. TNF-α-sensitive MCF-7 cells. MCF-7-MEK5 is characterized by 1) a morphological change consistent with epithelial-to-mesenchymal transition, 2) over expression of MEK5 (MAPK), 3) resistant to tumor necrosis factor-α and tamoxifen, and 4) estrogen independent. MCF-7-VEC represents a control cell line transfected with empty vector.
- We have identified seven protein spots that are differentially expressed in MCF-7-MEK5 and MCF-7-VEC using 2-D gel electrophoresis. Real time PCR analysis confirmed the findings in proteomics work.
- We have obtained evidence at both the gene and protein expression level, that MCF-7-MEK5 cells express EMT markers, suggesting involvement of MEK5 in the regulation of EMT in breast cancer cells.
- Wang's lab and Burow lab worked closely to revise the original manuscript, design and carry out additional experiments needed to improve the manuscript, and finalize the paper for publication in December of 2008. The manuscript has now appeared in the journal Breast Cancer Research: **Zhou C**, **Nitschke AM**, **Xiong W**, **Zhang Q**, **Tang Y**, **Bloch M**, **Elliott S**, **Zhu Y**, **Bazzone L**, **Yu D**, **Weldon CB**, **Schiff R**, **McLachlan JA**, **Beckman BS**, **Wiese TE**, **Nephew KP**, **Shan B**, Burow ME, Wang G. **Proteomic analysis of tumor necrosis factor-alpha resistant human breast cancer cells reveals a MEK5/Erk5-mediated epithelial-mesenchymal transition phenotype. *Breast Cancer Research*** 2008**;10(6):R105. Epub 2008 Dec 16.**
- As the proposed Program Director, Guangdi Wang prepared and submitted an institutional program proposal entitled "Xavier's RCMI Cancer Research Program" to NIH's NCRR division on January 25, 2009.
- Drs. Wang and Burow worked together to prepare a 4-year proposal for NIH titled "Proteomic and Phosphoproteomic Study of MEK5-Erk5 Signaling and Rapid Estrogen Signaling in Breast Cancer Cells". The proposal was submitted to NIH's SCORE program as an SC-1 type on January 25, 2009.
- A proposal for the DoD IDEA AWARD (oppW81XWH-08-BCRP-IDEA) was submitted in May, 2008, reviewed and not funded. The title: MEK5-Erk5 Signaling Regulates

Epithelial-to-mesenchymal Transition in Breast Cancer Progression.

- An NIH-R01 was submitted in 2008:"MEK5-Erk5 pathways in survival signaling and tumor progression to drug resistance" (M. Burow Principal Investigator) 25% effort Agency: NIH-NCI (BMCT), Type: 1**R01CA**125806-01A1, 07/01/09-06/30/14, $1,856,250 The long-term goal of this project is to identify the signaling pathways critical to the development of resistance to chemotherapeutics agents and the progression to a hormone independent phenotype in carcinoma of the breast. Submitted July 2008 - **(scored 219)**
- PCR Array gene array methods have identified pesticide mixtures that have potentiated gene regulation events in breast cancer cells when compared to treatment with one pesticide alone.
- The potential for small peptides to inhibit P23 and HSP90 interactions has been evaluated.
- Particular rare sugars have been shown to inhibit breast cancer cell proliferation and estrogen dependant reporter gene activity.
- All members of this research team are either now, or in the process of being appointed as adjunct faculty at Tulane.
- XU is now a member of the LCRC and has annula funds to continue cancer research development activities started by this DOD program, including the hire of faculty with cancer research focus.

Reportable Outcomes

A manuscript based on these findings has been poublished in the *Breast Cancer Research* in December of 2008:

Proteomic analysis of tumor necrosis factor-α resistant human breast cancer cells reveals a MEK5/Erk5-mediated epithelial-mesenchymal transition phenotype

Changhua Zhou, Ashley M Nitschke, Wei Xiong, Qiang Zhang, Yan Tang, Michael Bloch, Steven Elliott, Yun Zhu, Lindsey Bazzone, David Yu, Christopher B Weldon, Rachel Schiff, John A McLachlan, Barbara S Beckman, Thomas E Wiese, Kenneth P Nephew, Bin Shan, Matthew E Burow, Guangdi Wang

Conclusion

We have established four collaborative breast cancer research projects while at the same time we have built a framework of activities for XU faculty to utilize for interaction with the TCC/LCRC to develop cancer research initiatives involving Xavier undergraduate and pharmacy students. With Xavier a member of the LCRC, we will continue supporting the development of cancer research on campus through variety of mechanisms.

Request for No Cost Extension and Future Opportunities

In the no cost extension year, we plan to continue addressing project aims and evaluating data in preparation for publication and as preliminary data for research proposals.

We will continue building our small cancer research network at Xavier by holding regular cancer research discussion meetings, developing the aims of the NCI P20 grant funded in 2005 and our participation in the LCRC. Our long term goal is to establish a core of faculty at Xavier that are active in cancer research and education.

Continuing Challenges

The main obstacle in this program is the increased administrative loads of participating faculty. Dr. Wiese, PI of both the Xavier DOD Breast and Prostate programs is also the manager of the Xavier NCI P20 program and now Associate Director of the LCRC for Xavier.

References

NA

Appendices

Appendix

Louisiana Cancer Research Consortium
Monday Cancer Research Discussion Meetings at Xavier

Noon – 1:00pm
Room 420 Xavier College of Pharmacy
Lunch provided (11:45am)

Date:	Presenters
Monday September 22, 2008	Student Research Presentations (NCI fellows)
Monday October 6, 2008	Student Research Presentations (4)
Monday October 20, 2008	Shuh Project: Transcriptional regulation of the serum response pathway by the Human T-cell Lymphotrophic Virus Type I (HTLV-I) Tax protein.
Monday November 3, 2008	Johanson Project: Defining the role of FOXO1a in Pax3-FOXO1 DNA binding
Monday November 17, 2008	Biliran Project: "A potential role of anoikis effector Bit1 (Bcl-2 inhibitor of transcription 1) in tumorigenesis and metastasis"
Monday December 1, 2008	Bhattacharjee Project
Monday January 12, 2009	Wiese-Hill Project
Monday January 26, 2009	Parker-Johnson Project
Monday February 9, 2009	LCRC Gene Therapy/Dr. Wolfgang/Dr. Muniruzzaman
Monday March 2, 2009	Ireland-Mageed Project
Monday March 16, 2009	Foroozesh Project, presented by Dr. Jiawang Liu: "Synthesis of Ceramide Derivatives"
Monday March 30, 2009	Kolesnichenko Project
Monday April 13, 2009	Arora-Sikka Project
Monday April 27, 2009	Wang-Burow Project
Monday May 11, 2009	Mandal Project
Monday May 25, 2009	Stevens Project
Monday June 8, 2009	Zhang Project
Monday June 22, 2009	Wolfgang-Miller Project